

FORM PTO-1390 (Modified)  
(REV 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

294-102 PCT/US

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

09/869409 ✓

INTERNATIONAL APPLICATION NO.  
PCT/NL99/00804INTERNATIONAL FILING DATE  
24 December 1999PRIORITY DATE CLAIMED  
06 January 1999 ✓

TITLE OF INVENTION

Accessing Leaf and/or Stem Parts of Plants ✓

APPLICANT(S) FOR DO/EO/US

Anne Coenraad HULST, Jan Josef Maria Hubert KETELAARS, and Johan Pieter Marinus SANDERS ✓

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
  - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
  - b. ☒ has been communicated by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
  - a. ☒ is attached hereto.
  - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
  - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
  - b. ☐ have been communicated by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

## Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☒ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☐ Other items or information:

## EXPRESS MAIL CERTIFICATE

Date 06/25/01 Label No. EL470332425 US

I hereby certify that on the date indicated above, I deposited this paper or fee with the U.S. Postal Service & that it was addressed for delivery to the Assistant Commissioner for Patents, Washington, D.C. 20231 by "EXPRESS MAIL Post Office to Addressee" Service.

Name (Print) CASSANDRA LEMSSignature Cassandra Lems

25 JUN 2001

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.492(a)(1)) <b>09/869409</b>		INTERNATIONAL APPLICATION NO. <b>PCT/NL99/00804</b>		ATTORNEY'S DOCKET NUMBER <b>294-102 PCT/US</b>	
---	--	--	--	---	--

24. The following fees are submitted:

BASIC NATIONAL FEE ( 37 CFR 1.492 (a) (1) - (5)) :				CALCULATIONS PTO USE ONLY	
<input type="checkbox"/>	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .....	<b>\$1000.00</b>			
<input checked="" type="checkbox"/>	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .....	<b>\$860.00</b>			
<input type="checkbox"/>	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .....	<b>\$710.00</b>			
<input type="checkbox"/>	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) .....	<b>\$690.00</b>			
<input type="checkbox"/>	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) .....	<b>\$100.00</b>			
<b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>					
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).					
			<b>\$0.00</b>		
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	20 - 20 =	0	x \$18.00	<b>\$0.00</b>	
Independent claims	1 - 3 =	0	x \$80.00	<b>\$0.00</b>	
Multiple Dependent Claims (check if applicable).			<input checked="" type="checkbox"/>	<b>\$270.00</b>	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				<b>\$1,130.00</b>	
<input type="checkbox"/> Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				<b>\$0.00</b>	
<b>SUBTOTAL =</b>				<b>\$1,130.00</b>	
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).				<b>\$0.00</b>	
<b>TOTAL NATIONAL FEE =</b>				<b>\$1,130.00</b>	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).				<b>\$0.00</b>	
<b>TOTAL FEES ENCLOSED =</b>				<b>\$1,130.00</b>	
				<b>Amount to be: refunded</b>	<b>\$</b>
				<b>charged</b>	<b>\$</b>

- a. ☒ A check in the amount of **\$1,130.00** to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account No. \_\_\_\_\_ in the amount of \_\_\_\_\_ to cover the above fees. A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **08-2461** A duplicate copy of this sheet is enclosed.
- d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Ronald J. Baron, Esq.  
Hoffmann & Baron, LLP  
6900 Jericho Turnpike  
Syosset, New York 11791

Telephone: 516-822-3550  
Facsimile: 516-822-3582

  
SIGNATURE

Ronald J. Baron

NAME

29,281

REGISTRATION NUMBER

25 June 2001

DATE



13 Rec'd PCT/PTO 06 AUG 2001  
09/869409  
PATENT

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants: Hulst, et al.

Examiner: Unassigned

Serial No: 09/869,409

Group Art Unit: Unassigned

Filing Date: June 25, 2001

Docket: 294-102 PCT/US

For: ACCESSING LEAF AND/OR  
STEM PARTS OF PLANTS

Dated: August 1, 2001

Assistant Commissioner for Patents  
Washington, DC 20231

**PRELIMINARY AMENDMENT**

Sir:

Applicants respectfully submit the following Preliminary Amendment for entry in the above-identified application prior to examination.

**IN THE SPECIFICATION:**

On page 1, before line 1, please insert the following before the first paragraph:

**BACKGROUND OF THE INVENTION**

On page 6, line 16, please inset the following:

**SUMMARY OF THE INVENTION**

On page 8, before line 1, please insert the following before the first paragraph:

**DETAILED DESCRIPTION OF THE INVENTION**

**IN THE CLAIMS:**

Please cancel Claim 1 and insert therefor the following new Claim 19:

19. (New) A method for separating components from vegetable material having at least leaf and/or stem parts comprising:
- a) at least partially fiberizing said vegetable material and
  - b) subsequently separating said at least partially fiberized material into
    - i) a fiber fraction comprising relatively firm tissues, such as epidermis, sclerenchyma, and vascular bundles, and
    - ii) a juice stream comprising soft tissues, such as parenchyma and cytosol.

Please amend Claims 2, 3, 5 and 6 to read as follows:

2. (Amended) A method according to claim 19, wherein the juice stream comprises chloroplasts.
3. (Amended) A method according to claim 19, wherein the material is mechanically fiberized.
5. (Amended) A method according to claim 19, wherein the fiber fraction is separated from the juice stream comprising one of screening, centrifugation, processing by hydro(cyclone), centriscreening, decanting, sedimentation, or combinations thereof.
6. (Amended) A method according to claim 19, wherein the vegetable material originates from a cultivated crop.

Please cancel Claim 8-18 without prejudice.

Please add new Claims 20-41, as follows:

20. (New) A fiber fraction obtained by a method for separating components from vegetable material having at least leaf and/or stem parts comprising:
- a) at least partially fiberizing said vegetable material and
  - b) subsequently separating said at least partially fiberized material into
    - i) a fiber fraction comprising relatively firm tissues, such as epidermis, sclerenchyma, and vascular bundles, and
    - ii) a juice stream comprising soft tissues, such as parenchyma and cytosol.
21. (New) A biodegradable product comprising a fiber fraction obtained by a method for separating components from vegetable material having at least leaf and/or stem parts comprising:
- a) at least partially fiberizing said vegetable material and
  - b) subsequently separating said at least partially fiberized material into
    - i) a fiber fraction comprising relatively firm tissues, such as epidermis, sclerenchyma, and vascular bundles, and
    - ii) a juice stream comprising soft tissues, such as parenchyma and cytosol.
22. (New) A biodegradable product according to claim 21, wherein said product is paper.

23. (New) A biodegradable product according to claim 21, wherein said product is cardboard.
24. (New) A biodegradable product according to claim 21, wherein said product is fiberboard.
25. (New) A biodegradable product according to claim 21, wherein said product is used in the preparation of a moisture absorbing material.
26. (New) A biodegradable product according to claim 21, wherein said product is used in the preparation of growth media.
27. (New) A biodegradable product according to claim 21, wherein said product is a soil improver.
28. (New) A biodegradable product according to claim 21, wherein said product is fuel to produce energy.
29. (New) A juice stream obtained by a method for separating components from vegetable material having at least leaf and/or stem parts comprising:
- a) at least partially fiberizing said vegetable material and
  - b) subsequently separating said at least partially fiberized material into
    - i) a fiber fraction comprising relatively firm tissues, such as epidermis, sclerenchyma, and vascular bundles, and
    - ii) a juice stream comprising soft tissues, such as parenchyma and cytosol.

30. (New) A juice stream according to claim 29, which contains more than 55% of the crude protein of the vegetable material.
31. (New) A juice stream according to claim 30, which contains preferably more than 75% of the crude protein of the vegetable material.
32. (New) A juice stream according to claim 31, which contains preferably more than 90% of the crude protein of the vegetable material.
33. (New) A protein enriched product comprising a juice stream obtained by a method for separating components from vegetable material having at least leaf and/or stem parts comprising:
- a) at least partially fiberizing said vegetable material and
  - b) subsequently separating said at least partially fiberized material into
    - i) a fiber fraction comprising relatively firm tissues, such as epidermis, sclerenchyma, and vascular bundles, and
    - ii) a juice stream comprising soft tissues, such as parenchyma and cytosol.
34. (New) A protein enriched product according to claim 33, wherein said product is food.

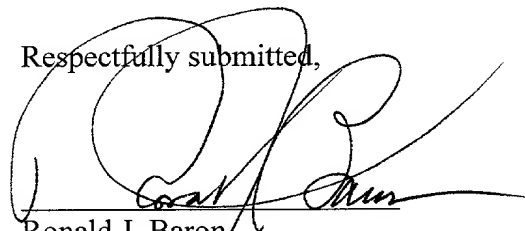
35. (New) A protein enriched product according to claim 34, wherein said food is for human nourishment.
36. (New) A protein enriched product according to claim 34, wherein said food is for use in livestock feeding.
37. (New) A protein enriched product according to claim 33, wherein said product is a substrate for fermentation.
38. (New) A protein enriched product according to claim 33, which results from recovering or purifying a substance contained in said juice stream.
39. (New) An apparatus for separating components from vegetable material having at least leaf and/or stem parts comprising:
- a) a fiberizer which dissociates relatively firm tissues from the relatively soft tissues of said vegetable material, and
  - b) a separator which separates the fiber fraction from the juice stream.
40. (New) An apparatus according to claim 39, wherein said apparatus comprises a refiner.
41. (New) An apparatus according to claim 39, wherein said apparatus comprises a blender.



**REMARKS**

Applicants have undertaken to cancel Claims 1 and 8-18, amend Claims 2, 3, 5, and 6, and add new Claims 19-41 in the above-identified application in order to remove improper multiple dependencies and conform to U.S. practice. No new matter has been added. In addition, headings have been added to the specification. Accordingly, entry hereof and examination on the merits are respectfully requested.

Respectfully submitted,



Ronald J. Baron  
Registration No. 29,281  
Attorney for Applicant(s)

HOFFMANN & BARON, LLP  
6900 Jericho Turnpike  
Syosset, New York 11791  
(516) 822-3550



Application No. 09/869409  
Filing Date: June 25, 2001  
Docket No.: 294-102 PCT/US  
Page 8

**VERSION OF AMENDMENT WITH MARKINGS TO SHOW CHANGES MADE**

**IN THE SPECIFICATION:**

On page 1, before line 1, please insert the following before the first paragraph:

**BACKGROUND OF THE INVENTION**

On page 6, line 16, please inset the following:

**SUMMARY OF THE INVENTION**

On page 8, before line 1, please insert the following before the first paragraph:

**DETAILED DESCRIPTION OF THE INVENTION**

**IN THE CLAIMS:**

Please amend Claims 2, 3, 5 and 6 to read as follows:

2. (Amended) A method according to claim 19 [1], wherein the juice stream comprises  
chloroplasts.
3. (Amended) A method according to claim 19 [1 or 2], wherein the material is mechanically  
fiberized.

5. (Amended) A method according to claim 19 [any one of claims 1-4], wherein the fiber fraction is separated from the juice stream [through] comprising one of screening, centrifugation, processing by hydro(cyclone), centriscreening, decanting, sedimentation, or combinations thereof.
6. (Amended) A method according to claim 19 [any one of claims 1-5], wherein the vegetable material originates from a cultivated crop.

Title: Accessing leaf and/or stem parts of plants.

The invention relates to the separation and recovery of components from vegetable raw materials.

Plants, like most organisms, are made up of cells. A plant cell consists of a lipid membrane with a generally aqueous content, the cytosol, which contains the various cell organelles (likewise surrounded by lipid membranes), such as nucleus, mitochondria, endoplasmic reticulum and chloroplasts, and the cytoskeleton, made up of microfilaments and microtubules, which gives the cell an inner structure. Also present in the plant cell are vacuoles which play an important role in keeping the plant cell under tension; the vacuoles maintain the turgor of the cell.

The constituent components of a plant cell can be roughly distinguished into water, which accounts for the greater part by far of a living cell, components such as salts, (precursors of) lipids, carbohydrates, amino acids and nucleotides, macromolecules such as starches, proteins and nucleic acid and a multiplicity of other molecules, including vitamins and pigments such as chlorophyll, carotene and xanthophyll.

A plant cell is generally surrounded by a cell wall which provides firmness and structure to the plant tissue. The cell wall is mainly built up from (hemi)cellulose and other carbohydrate polymers, which have aggregated to fibers. Woody plants further contain an ample amount of lignin, a polymer made up of phenols and other aromatic monomers.

Plant tissue is made up of plant cells, all of which, when living, basically satisfy the above description. An important distinction can be made between relatively firm tissues which comprise virtually no chloroplast or other plastid containing cells, and the relatively soft tissues which generally do. Tissues which generally comprise no chloroplast containing cells are, for instance, the epidermis or skin tissue of a plant, the collenchyma and sclerenchyma or stroma of a plant and the vascular

fiber bundles or the vascular tissue, comprising the important transport vessels (wood vessels and sieve tubes) in the plant. When a part of a plant is strongly lignified, in general, over time, the majority of the cells in the lignified part die off and only residues of the cell content are left. In particular the cytosol and the organelles present therein are lost, but the vascular fiber bundles, skin and stromas generally give the plant form and structure and generally remain present when the plant is dead.

Characteristically, these relatively firm tissues (in particular vascular bundles, sclerenchyma and epidermis) comprise no to virtually no chloroplast containing cells, while an important part (at least in the aerial leaf and stem parts of the plant) of the relatively soft tissues, also called chlorenchyma, is made up chiefly of only chloroplast-containing parenchymal cells; indeed, this is where photosynthesis occurs.

It has long been known to recover various components from vegetable raw materials for further use in, for instance, food for human or feed for animal consumption through mechanical methods. Often, plants are merely comminuted or chopped to make them suitable for consumption, an example being the chopping of maize for feed.

However, in particular the components present in the plant cell cytosol are outstandingly suitable for human food or animal feed, since these can be building materials for corresponding components which are found in animal cells.

Mechanical processing is applied, for instance, to feed crops, such as grass, lucerne and other fresh and green harvested plants which, often as virtually whole plant, in particular the leaf and/or stem parts and in most cases not including the roots, are used for recovering, for instance, (animal) feed components. Such vegetable raw materials are generally recovered through pressing of (preferably chopped or otherwise comminuted) leaf and/or stem material, whereby a part of the vegetable material is obtained as press juice, while the residual and pressed material is known as press cake.

5 The pressure forces exerted by pressing generally result in the opening up (snapping or bursting) of plant cells in the material, so that the aqueous but food component-rich cytosol, possibly with residues of the organelles and the lipid membrane surrounding the cell, is liberated from the cell as press juice. Press juice is generally treated further, for instance through screening, whereafter, for instance, the protein in the juice is recovered by means of coagulation through, for instance, acid and/or heat treatment. Press juice may also be further processed through (ultra or membrane) filtration, drying, fermentation or other methods known to the skilled person. Protein-rich or otherwise high-grade nutrients for human and animal consumption, but also pigments such as carotene (provitamin A), can be recovered from cytosol in this way.

10 The resultant, relatively dry press cake is generally considered to be less rich in food; it contains relatively intact fiber bundles composed of (not directly) digestible cellulose fibers, adherent press juice and residual plant cells which have not been accessed under the influence of the pressing. Especially these residual plant cells with unrecovered cytosol give fodder value to the press cake, which is generally dried and, pelleted or otherwise, is used as relatively low-grade roughage component in fodders, in particular for ruminants.

20 For mechanically accessing, for instance, lucerne or grasses, traditionally a method is used which is based on the disintegration of the vegetable raw material by means of hammer mills followed by squeezing the disintegrated raw material (here designated as pulp) using screw presses or belt presses. The pulp is thereby separated into a press cake fraction and a press juice fraction. The juice fraction is regarded as the fraction in which the industrially recoverable content substances from the plant material are contained. Hammer mills typically consist of a rotor on which fixed or freely movable elements are disposed which upon rotation of the rotor are brought into contact with the vegetable raw material and disintegrate it through force of impact. The disintegratory effect of hammer mills is relatively large when the vegetable material has a good

turgor, i.e. when the plant cells are under tension. In that case, the force of impact causes the tissue to snap and causes the cell content constituents to be liberated with the tissue fluid. If turgor is low, beating the plant material will cause it to be compressed. The tissue then remains more or less intact and the result is that the cell content becomes available to a much lesser extent. This has major consequences for the recoverability of particularly those cell content constituents that are present in the vegetable biomass only partly in dissolved form and for another part in the form of solid, undissolved matter. This holds true inter alia for vegetable proteins, but also for lipids and pigments. Also known (for instance from US 5,464,160) are hammer mills where relatively dry material is separated into two fractions, this while neglecting the so valuable juice stream with protein-rich cytosol. Accordingly, these types of mills are not suitable for processing fresh, relatively wet material and eventually produce a relatively wet fiber fraction.

In the above-described methods of pressing vegetable material which contains at least leaf and/or stem parts, it is generally of importance that the material be processed while still as fresh as possible, shortly after harvesting. Only then are the plant cells sufficiently under tension to be able to burst or snap under pressure so that the cytosol is liberated. When, after harvesting, already some time has elapsed before the plant parts are pressed, they are already dried out to some extent by then, the plant cells present have lost a large part of the necessary turgor and are too slack to be able to snap or burst under pressure. Accordingly, in non-fresh material, the recovery of press juice will proceed with less efficiency. The same holds for material stemming from plants which, even before they were harvested, already lost a large part of the turgor in their plant cells through drying out and/or maturation. In general, such plants are not (completely) green anymore but acquire brown or yellow aspects. Lignified plant parts are altogether ineligible for the above methods, since most cells have died off in them, or in any case they contain only a very

In general, plant material is separated into a press cake fraction and a press juice fraction. Characteristic of this method is the only partial

The press juice consists substantially of the aqueous content of cells: the vacuole content and the cytoplasm having therein cell organelles such as chloroplasts in intact or disintegrated form; cell wall constituents are substantially absent in that they remain behind in the press cake.

The traditional method of fractionation has as a consequence that upon squeezing the pulp, only a part of the cell content constituents end up in the juice stream and another part remains behind in the press cake. Accordingly, the press cake still contains, in addition to the greater part of the cell walls, a part of the cell content constituents and, by virtue of that, is used as fodder.



The existing pressing methods for separating high-grade from low-grade components from vegetable material are therefore relatively strongly dependent on the turgor of the cells present in the vegetable material, which limits the application of these methods to their application to relatively fresh and green material. Often, the resultant press cake, also when fresh and/or green material is used, still contains large amounts of unaccessed plant cells with high-grade cytosol in them, while only a low price can be obtained for press cake since it is in fact suitable only as a relatively low-grade component of animal fodder. For recovering high-grade components from leaf and/or stem parts, there is a need for better methods which can access the plant cell with a higher efficiency than the existing methods, can make the cytosol fraction more available for recovery, and affords better marketing possibilities for the fiber-containing residual material. The object of the invention is to provide for this need.

The invention provides a method for separation of components from vegetable material, such as leaf and/or stem parts, characterized in that the material is at the least partly fiberized and subsequently is separated into a fiber fraction and a juice stream, such that the fiber fraction chiefly comprises relatively firm tissues such as epidermis, sclerenchyma and vascular bundles, and the juice stream chiefly contains soft tissues such as parenchyma, and cytosol. In a preferred embodiment, the invention provides a method for separating a juice stream comprising in particular parenchyma with chloroplasts.

The invention provides a new method of fractionation which consists of at least two steps: a first step in which the vegetable material is fiberized through the action of shear forces and a second step in which the fiber fraction is separated from the rest. Fractionation of vegetable biomass means the separation into a number of fractions. By fractionating biomass, new product streams are formed with other application possibilities than the raw material itself. Consequently, these new product

streams jointly often represent more value than the original biomass. The invention provides a new technique which is based on fiberization and subsequent defibration of vegetable biomass.

In a preferred embodiment, the invention provides a method for  
5 separation of components from vegetable material, characterized in that the material is at the least partly mechanically fiberized and subsequently is separated into a fiber fraction and a juice stream, with the fiber fraction (see, for instance, Figs. 1 and 2, also for a comparison with a traditional method) principally comprising relatively firm tissues such as epidermis,  
10 sclerenchyma and vascular bundles, and the juice stream (see, for instance, Figs. 6 and 7, also for a comparison with a traditional method) principally containing soft tissues such as parenchyma, and cytosol. The mechanical fiberization is effected, for instance, through treatment of the material in a blender. Preferably, certainly when application on an  
15 industrial scale is desired, the fiberization is done, according to the invention, with an apparatus such as a (pressure) refiner, with grinding disks, such as employed in the pulp and paper industry, or in an apparatus of equivalent action by which the vegetable material can be fiberized to enable separation into a fiber fraction which principally  
20 comprises relatively firm tissues such as epidermis, sclerenchyma and vascular bundles, and the juice stream principally comprising soft tissues such as parenchyma, and cytosol.

The method according to the invention is applicable to all fiber containing vegetable materials, originating both from cultivated plants  
25 (crop plants) and from wild plants, as well as to crossing products of cultivated plants among themselves or with wild plants. Examples are: vegetable biomass originating from cultivated grassland, but also from natural grounds and roadsides, feed crops such as forage grasses and corn, lucerne, clover, and other papilionaceous plants, fiber crops such as flax  
30 and hemp, and the tops of crops normally grown solely for their seeds, fruits or tubers, such as grains, beets, peas, beans, potatoes, carrots, cassava, sweet potato.

In fiberization, the vascular tissue with the sclerenchyma and the epidermis (jointly the fiber fraction) is mechanically dissociated from the other, substantially parenchymal tissue. This parenchymal tissue is at the same time accessed and the cell content constituents from it thereby become available substantially completely. Fiberization can be done using refiners such as they are in use in the pulp and paper industry for fiberizing wood and wood pulp. Refining, or fiberization, typically occurs with addition of moisture to the plant material. The result is then a slurry of fiberized material from which the fibers can be removed. The fiber fraction (fiber stream) which is thus recovered, is suitable, through its nature and composition, inter alia for the following applications: as raw material for paper and cardboard (solid cardboard, folding cardboard and form cardboard), as raw material for the production of fiberboard materials (softboard, hardboard, chipboard, MDF, HDF and MDF/HDF form parts) and composites, as raw material for moisture absorbing materials, such as diapers, sanitary napkins, and so forth, as raw material for the preparation of growth media (potting compost and substrates), mulches (as protection against erosion, and as weed and disease suppressant), as soil improver or as fuel.

In defibration, the liberated fiber is separated, for instance through screening, from the other plant constituents. Through washing and screening, the fiber can be further purified and as many non-fiber constituents as possible can still be recovered with the washing water. The defibered slurry then consists of a mixture of added water, tissue fluid, cell content constituents and finely dispersed cell walls coming from the parenchymal tissue. From the defibered slurry or juice stream, content substances can be recovered in a more or less pure form, such as: proteins, peptides and amino acids, enzymes, pigments, lipids, fatty acids, starches, soluble sugars and (cell wall) carbohydrates for use in livestock feeding, human nourishment, or as substrate for fermentations, or, through concentration, fodder or food products can be made with a high nutritive value as a result of the removal of the non-digestible or poorly digestible

fiber fraction. The defibered slurry can be further fractionated in subsequent steps. One possibility is, for instance, the separation of all solid parts through centrifugation, which may or may not be preceded by a coagulation step through heating, acidification or otherwise. Another possibility is to convert the parenchymal cell walls into soluble sugars using cell wall splitting enzymes (pectinases, cellulases, etc.) and thus adding them to the fraction of dissolved substance in the defibered slurry.

Characteristic of the method as provided by the invention is the split at tissue level into a fiber fraction which contains the relatively firm tissues (vascular bundles, sclerenchyma and epidermis) and a defibered fraction which contains the relatively soft tissues (parenchyma) with their content. Briefly summarized, the difference between the traditional and novel method is the extraction of tissue fluid (traditional) versus tissue fractionation (new method).

The invention also provides an apparatus for practicing a method according to the invention. Such an apparatus is characterized by means suitable for the fiberization according to the invention, whereby the relatively firm vascular tissue with, for instance, the sclerenchyma and the epidermis (jointly the fiber fraction) is mechanically dissociated from the other, substantially parenchymal tissue. This parenchymal tissue is at the same time accessed and the cell content constituents therefrom thereby become available substantially completely. 'Fiberization' is herein understood to mean that the plant material is exposed to such forces that the relatively firm tissues are dissociated virtually completely from the relatively soft tissues. As a resultant of the forces which effect this fiberization, the great majority, if not virtually all, of the plant cells will be accessed, so that the cytosol is liberated. This cytosol, as a juice stream generally also including residues of the organelles and the cell surrounding lipid membrane and parenchymal cell walls, can be relatively simply separated from the fiber component through screening or through other separation means known to those skilled in the art.

A first advantage of the invention is that the efficiency of the method is not dependent on the turgor of the plant cells present in the material, so that the plant cells can be accessed with greater efficiency than is conventional in the above-described pressing methods.

5 A second advantage of the invention is that the invention provides two product streams which as such are very pure. A first one, the fiber fraction, contains principally cellulose and hemicellulose, principally consisting of the elements C, H and O (which in itself yields advantages for a clean combustion); a second one contains all valuable and complex  
10 content substances to be found in the parenchyma and cytosol, and which can be further separated relatively simply.

The two product streams can be separated from each other by, for instance, screening. Other separation methods than screening are also conceivable, for instance centrifugation, processing by (hydro)cyclone and  
15 centriscreening, and decanting or sedimentation, or combinations of these methods. In defibration, the liberated fiber is separated from the other plant constituents through, for instance, screening. By washing and screening, the fiber can be further purified and as many non-fiber constituents as possible can still be recovered with the washing water. The  
20 defibered slurry then consists of a mixture of added water, tissue fluid, cell content constituents and finely dispersed cell walls coming from the parenchymal tissue.

A first product stream as contemplated by the invention is a (generally nutritionally high-grade) juice stream consisting of an aqueous  
25 solution/suspension of virtually all high-grade components or nutrients from the vegetable material (such as sugars, fructose-oligosaccharides, proteins, lipids, pigments, and the like). Through removal of the (nutritionally low-grade) fiber components, there is formed (on a dry matter basis) this relatively high-grade product stream, from which the  
30 various components can be further isolated relatively simply. The defibered product or the juice stream consists substantially of parenchyma, partly as intact cells, partly as disintegrated cell material.

The color of the defibered product is typically green due to the presence of intact or broken chloroplasts, sometimes brown-green through browning during the fractionation. Macroscopically, it is a liquid. Microscopically, principally intact and disintegrated parenchyma cells and cell organelles  
5 such as chloroplasts are visible in this liquid.

The second product stream, the fiber fraction as contemplated by the invention, consists of the relatively hard tissues. These are typically the vascular bundles, the sclerenchyma and the epidermis. The cell content is absent from these tissues or is removed virtually completely  
10 during fractionation and washing. Consequently, fiber consists predominantly of cell wall components. Chloroplasts are virtually absent in a pure fiber preparation. The color of the washed fiber typically varies from white to yellow or light-brown. Sometimes, a light-green color may arise as a result of impregnation with chlorophyll during recovery.

Macroscopically, the fiber fraction has a fiber structure principally through the filamentary character of the vascular bundles. Microscopically, in addition to the filamentary structures of vascular bundles and sclerenchyma, typically, pieces of epidermis tissue are also recognizable, consisting of sheets one cell layer thick. The vascular  
20 bundles are built up from several cells including wood vessels and sieve tubes. Depending on the extent of fiberization, fibers consisting of one cell occur too, and further the residues of cell walls and (spiral, reticulate or ring-shaped) cell wall thickenings. Typical of the epidermis sheets is the presence of stomata and silicious teeth or hairs.

25 The fiber stream as contemplated by the invention consists substantially exclusively of a wet solid fiber stream (chiefly cellulose and hemicellulose) basically having no nutritive value, since this fraction is not directly, and microbiologically only to a slight extent, digestible. However, the absence of digestibility makes it possible to use the fiber  
30 stream for non-food applications, this in contrast to, for instance, the press cake coming from the above-described traditional pressing methods where the press cake is in fact suitable only for fodder applications and would

soon rot if it were not prepared into food and was eaten or further preserved.

For example, the invention provides the use of a fiber fraction for the production of energy. The fiber fraction contains principally the

5 carbohydrates cellulose and hemicellulose (composed principally of the elements C, H and O), which are eminently combustible and hence can be converted with a high efficiency to useful energy in, for instance, a combined heating and power station, and which may be expected to entail no or minor emission of harmful substances upon combustion. Processing

10 plant material according to a method as contemplated by the invention, followed by the use of the resultant fiber fraction as fuel, will contribute to the reduction of the CO<sub>2</sub> emission, since what is involved here is a non-fossil fuel. Also, as such, the combustion of the fiber fraction will be cleaner for the environment, since the fiber fraction is hardly, if at all,

15 contaminated with the salt residues (such as K, Na, Cl, P compounds) and protein residues (which include S and N compounds) normally occurring in dry plants. These salt residues and protein residues, coming from the cytosol, have been separated, along with the juice stream, from the fiber fraction. Combustion of the fiber fraction (having therein principally C, H

20 and O compounds which are converted by combustion to H<sub>2</sub>O and CO<sub>2</sub>) will therefore entail a much lesser environmental impact than combustion of other plant material in which all these salt residues and protein residues are still present. Protein combustion contributes in particular to the emission of sulfur and nitrogen compounds such as sulfur and nitrogen

25 oxides, and incombustible salt residues will contribute to the residual ash volume. Upon combustion of a fiber fraction according to the invention, the emission of, for instance, sulfur and nitrogen oxides, and the residual ash volume having therein the salt residues will be much smaller.

Since the fiber material is of organic origin, it is also applicable, for

30 instance, as a peat substitute in, for instance, potting soil or in horticultural substrates.

In a preferred embodiment of the invention, the plant material is fiberized to such an extent that, for instance, the fiber material consists principally of elemental fibers, so that the so obtained fiber component or fiber stream is suitable, for instance, for further processing into cardboard and/or paper, or can be used as (natural) fiber in composites together with and in reinforcement of (artificial) resins.

Examples of vegetable material that can be treated with a method according to the invention are known (fodder) crops such as grasses (grains such as wheat, rye and maize included), lucerne, hemp, but also harvest residues of crops whose leaf and/or stem parts are normally not processed, such as potato or (sugar) beet tops which are generally left behind in the field upon harvesting; or crops which are generally not processed into juice, or are so processed on a limited scale only, such as spinach, lettuce and grass. The high efficiency of a method according to the invention renders the processing of such vegetable materials profitable.

The juice stream of plant materials processed according to a method contemplated by the invention is further treated, for instance through screening, whereafter, for instance, the protein, peptides, amino acids, and other components or content substances in the juice are recovered by, for instance, coagulation through, for instance, acid and/or heat treatment. The juice stream may also be further processed by (ultra or membrane) filtration, drying, fermentation, or other methods known to those skilled in the art. Protein-rich or otherwise high-grade nutrients for human and animal consumption, but also pigments such as carotene (provitamin A) can be recovered from cytosol in this way, also from that of leaf and/or stem parts.

Also eligible for processing in a method according to the invention is vegetable material not belonging to cultivated crops in the strict sense of the word, such as roadside grass mown along roads or highways, or mixtures of grasses and other wild plants mown in natural areas.



The invention further provides a method for separating components from vegetable material which has been harvested a relatively long time ago and has already, at least partly, dried out, or which can no longer be qualified as fresh and green, but has acquired a more woody and/or dry character for instance through maturation. Such material is not suitable for processing in a pressing method, but is now outstandingly processable, since the extent of turgor of the plant cell to be accessed is not important when a method according to the invention is used.

The invention provides a refiner, or an apparatus of comparable action, and the use of such an apparatus, for instance for separating components from vegetable material which does not (yet) exhibit any lignification, or exhibits only a minor extent of lignification, and in which parenchyma is present. This parenchyma with the cytosol present therein is the basis of the juice stream as contemplated by the invention. A refiner is generally used to break down wood chips into fibers for the purpose of making pulp for the production of paper and/or cardboard. The invention provides for the processing by means of a refiner, of a crop selected from a large variety of crops which traditionally have not been processed with a refiner. Refiners are generally not used for fresh and/or green material, since wood consists principally of dead or lignified tissue from which most parenchyma, with chloroplasts, has disappeared. Different types of refiners are known to those skilled in the art. There are, for instance, refiners with conical disks or with flat disks. The invention provides for the use of both types, and/or equivalent apparatuses, for instance convex/concave type composite grinding disks, in a method provided by the invention.

The invention will now be further explained in the experimental section of the description, without limiting it.

Experimental section

Experimentally, the invention was compared with the traditional technique. This was done using a lab(oratory) protocol and industrial equipment. On the basis of that, the nature of the fiber fraction can be assessed and the recoverability of content substances in the two methods  
5 can be compared. Results shown hereinbelow illustrate the difference in the recoverability of protein and other content substances.

#### Traditional method

In the experiments on a laboratory scale, the traditional method of  
10 grinding and pressing was simulated by pulping material in a Tecator Homogenizer and squeezing the pulp using an adapted draw pressure bench of Lloyd Instruments. It was provided with a cup having a perforated bottom plate (surface 50 cm<sup>2</sup>) in which 100 g of fresh pulp were pressed for 15 minutes at a pressure running up to 10 bar. The original  
15 material and the press juice were analyzed for nitrogen content, and the recoverability of protein was calculated as the amount of crude protein (amount of nitrogen multiplied by 6.25) in juice expressed as a percentage of the amount of crude protein in the original material.

20 On a larger scale, a hammer mill of the type Jenz A30 was employed to disintegrate grass and the thus obtained grass pulp was squeezed in a Vetter screw press with a compression ratio of 1:7.65 and a perforation of the cylinder wall of 0.7 mm. By passing plant material through the hammer mill a single time or several times, the material could be  
25 disintegrated to a greater or lesser extent.

#### New method

In the experiments on a laboratory scale, the new method was simulated  
30 by fine-chopping fresh grass in a cutter, then mixing 30 g of fine-cut grass pieces with 400 ml of water, and fiberizing same in a blender (Braun mixer) for 10 minutes, screening the slurry from the blender on an 850

micron screen, and washing and drying the screened-off fiber fraction. The fiber was analyzed for contents of nitrogen, ash and cell walls and thus the composition of the defibered slurry was calculated. The fiber yield was determined as the amount of dry matter in the fiber fraction as a  
5 percentage of the amount of dry matter in the starting material. The recoverability of protein was calculated as the amount of crude protein in the defibered slurry expressed as a percentage of the amount of crude protein in the original material.

10 The new method was also tested with a Sprout-Waldron 12 inch pressure refiner, with grinding disks of the type D2A505. Refining or fiberizing fresh grass was done under atmospheric conditions at a disk distance of 0.04 mm, with addition of water to a consistency of about 2% dry matter. The fiber was then screened on a screen with 140 micron openings.

15 The new method was also tested on a semitechnical scale using a Sunds Disk Refiner type RO 20 FLUFF serial no. 3838, year of manufacture 1985, provided with grinding disks with a high or low resistance to throughput. With this refiner, inter alia the effect of disk type and disk  
20 distance on throughput and fiber composition was investigated.

Refining occurred under atmospheric conditions with chopped grass, with or without addition of water. The fiberization of potato tops was also tested.

25 The grass originated from both cultivated grassland and natural grounds and was processed in fresh, chopped form. Samples of the fiberized material were rinsed by hand and screened and analyzed for nitrogen and ash content. The recoverability of crude protein was calculated on the  
30 basis of an average fiber proportion of 33% of the grass dry matter.

The potato tops originated from starch potato plants during the full growth phase of the potato plant. The tops were pulled mechanically and were consequently crushed to some extent. The potato tops consisted of stems and leaves. The potato tops, without prior washing, were processed

5 with the refiner while fresh, without addition of water. The fiberized material was squeezed by hand.

PCT/NL99/00804

## Experimental results:

### Description of the figures

#### 5 Figure 1 and Figure 2 (detail)

Press cake of grass (left) and grass fiber (right) stemming from perennial ryegrass (*Lolium perenne*).

10 In the press cake, the green color due to the presence of chloroplasts is conspicuous. Also, leaf fragments are recognizable by their size (cross section greater than 1 mm) and the characteristic ribs on the top of the leaf. The grass fiber is distinguished by the light color (virtually complete absence of chloroplasts), the filamentary structure and the small diameter of the individual fibers (in this case very much smaller than 1 mm). The distance between successive numbers is 1 cm.

15

#### Figure 3

Suspension of grass fiber from perennial ryegrass (*Lolium perenne*).

20 Visible are fibrous structures (vascular bundles) of a diameter of a few tens of micrometers and epidermis sheets of a smallest diameter of up to a few hundreds of micrometers.

#### Figure 4

25 Microscopic recording of epidermis in grass fiber originating from perennial ryegrass (*Lolium perenne*).

30 Characteristic is the presence of stomata in perennial ryegrass, concentrated in the epidermis of the top of the leaf. The more compact tissue on the side of the stomata is underlying sclerenchyma. The elongate epidermis cells have a cross section of about 20 micrometers.

### Figure 5

Microscopic recording of vascular bundles in grass fiber originating from perennial ryegrass (*Lolium perenne*).

- 5 Characteristic of vascular bundles are their being built up from several cells and the presence of vessels with reticulate thickenings. The diameter of the fiber in the middle of the figure is about 50 micrometers.

### Figure 6

- 10 Microscopic recording of parenchyma cells in the juice stream of defibered grass originating from perennial ryegrass (*Lolium perenne*). This juice stream belongs to the fiber fraction of Figures 1 and 2.

Characteristic of parenchyma cells in grass leaves is the abundant presence of chloroplasts. Some parenchyma cells, however, have been

- 15 broken during fractionation: only the cell wall is still visible, the chloroplasts occur in isolation in the surrounding fluid. The size of these parenchymal cells is about 20 \* 40 micrometers. The fraction shown in this figure was diluted prior to being photographed to bring out the relatively large amount of parenchyma cells in the juice stream according  
20 to the invention.

### Figure 7

Microscopic recording of parenchyma cells in press juice from grass originating from perennial ryegrass (*Lolium perenne*).

- 25 This press juice belongs to the press cake of Figures 1 and 2. The fraction shown in this figure was concentrated prior to being photographed to bring out the relatively small amount of parenchyma cells in the press juice.

30

### Figure 8

Process diagram for fiberizing or refining grass.

Figure 9

Process diagram for fiberizing or refining grass.

5 Figure 10

Process diagram for fiberizing or refining grass.

FIG. 9

## Fiberization

Table 1. Fiber composition and fiber yield of cultivated grasses, by species and variety, on average during the season, and of a few other crops.

5

Species/variety	Nitrogen content (g/kg dm <sup>**</sup> )	Ash content (g/kg dm)	Cel wall content (g/kg dm)	Fiber yield % of dry matter in raw material
Grasses				
<i>Lolium perenne</i> 4n Vr.*	4.0	50.6	867	28
<i>Lolium perenne</i> 2n Vr.	4.3	43.5	865	34
<i>Lolium perenne</i> 4n Lt.	4.5	41.1	879	29
<i>Lolium perenne</i> 2n Lt.	5.4	34.7	857	29
<i>Lolium multiflorum</i> 4n	3.8	47.4	877	24
<i>Lolium multiflorum</i> 2n	4.4	36.6	880	27
<i>Phleum pratense</i>	4.3	39.8	862	30
<i>Festuca arundinacea</i>	4.4	36.7	867	29
<i>Dactylis glomerata</i>	5.1	42.0	873	32
<i>Festuca pratensis</i>	4.5	44.2	872	32
Other plant materials				
Lucerne	5.7	18.9	824	28
Potato tops young	4.2	26.1	836	16
Potato tops old	3.7	50.7	714	21
Pea tops	4.8	25.7	832	29
Beet tops	12.0	79.7	680	9

\*) 4n = tetraploid; 2n = diploid;

Vr. = early-flowering; Lt. = late-flowering

10 \*\*) dm: dry matter



Fiberizing vegetable biomass yields a fiber fraction which, depending on the nature of the material, can vary from less than 10% to more than 30% of the dry matter. The exact number is also dependent on the mesh of the screen with which the fiber is separated and the intensity of washing. The fiber fraction in the case of *Lolium perenne* typically consists for more than 80% of cell wall material and has a nitrogen content mostly lower than 6-8 g per kg of dry matter and an ash content mostly lower than 50-100 g per kg of dry matter.

Table 2. Composition of fiber

		refiner	lab protocol
Ash	(g/kg d.m.)	22.3	26.0
Nitrogen	(g/kg d.m.)	5.3	4.4
Cell walls	(g/kg d.m.)	808	792

The composition of the fiber fraction is comparable for the experiments with the refiner and the experiments according to the lab protocol.

## Defibration

Table 3. Composition of grass and of the defibered grass slurry.

		Grass	Defibered slurry	
			refiner	lab protocol
Ash	(g/kg d.m.)	92.6	138	139
Nitrogen	(g/kg d.m.)	31.0	47.4	48.7
Cell walls	(g/kg d.m.)	544	375	438

In addition to the cell content constituents (such as protein), the defibered slurry also contains a part of the cell walls from the plant material. These are substantially the cell walls from the soft parenchymal tissue which disintegrate upon fiberization and subsequently, in defibration, pass the screen as finely dispersed material. The amount present in the defibered slurry is partly dependent on the diameter of the screen orifices.

Table 4. Recoverability of crude protein from cultivated grasses, by species and variety, on average during the season, and of a few other plant materials, upon grinding+pressing and upon defibration.

Species/variety	Grinding+pressing (%)	Defibration (%)
Grasses		
<i>Lolium perenne</i> 4n Vr.	30	95
<i>Lolium perenne</i> 2n Vr.	23	94
<i>Lolium perenne</i> 4n Lt.	22	95
<i>Lolium perenne</i> 2n Lt	16	94
<i>Lolium multiflorum</i> 4n	41	96
<i>Lolium multiflorum</i> 2n	35	95
<i>Phleum pratense</i>	11	94
<i>Festuca arundinacea</i>	21	94
<i>Dactylis glomerata</i>	31	93
<i>Festuca pratensis</i>	17	94
Other materials		
Lucerne	52	95
Potato tops young	51	98
Potato tops old	42	95
Pea tops	16	95
Beet tops	24	95

Defibrination yields a slurry mostly containing more than 70%, and preferably more than 80% or 90%, of all crude protein from the vegetable material. This protein can be recovered from it by centrifugation, which may or may not be preceded by heat coagulation.

5

In the traditional method of fractionation, the recoverability of crude protein is mostly less than 50%.

Table 5. Comparison of protein recoverability from grass upon repeated passage through hammer mill followed by pressing in a screw press, and upon fiberization according to the invention.

Protein recoverability	
(%)	
Hammer mill+screw press	
Passages through hammer mill	
1x	28
2x	30
4x	35
8x	43
Fiberization	93-96
according to the invention	

Even upon repeated disintegration of grass in a hammer mill followed by pressing in a screw press, the protein recoverability was found to be less than half of the protein recoverability measured upon fiberization of grass.

30

The results of the tests with the Sunds Disk Refiner are summarized in Table 6.

Choice of plate type and disk distance determine the extent of fiberization but determine protein recoverability only to a slight extent. A high throughput was possible in combination with a high protein recoverability (in this case >85%) both with protein-rich cultivated grass and with  
5 protein-low natural grass.

Potato tops are well processable with the refiner. In the fiber fraction the content of woody fibers is relatively high because the original potato tops consisted not only of leaf tissue but also of stem tissue. The high ash  
10 content in the fibers of the potato tops was caused to an important extent by the high sand content in the tops due to not washing the raw material.

11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77  
78  
79  
80  
81  
82  
83  
84  
85  
86  
87  
88  
89  
90  
91  
92  
93  
94  
95  
96  
97  
98  
99  
100  
101  
102  
103  
104  
105  
106  
107  
108  
109  
110  
111  
112  
113  
114  
115  
116  
117  
118  
119  
120  
121  
122  
123  
124  
125  
126  
127  
128  
129  
130  
131  
132  
133  
134  
135  
136  
137  
138  
139  
140  
141  
142  
143  
144  
145  
146  
147  
148  
149  
150  
151  
152  
153  
154  
155  
156  
157  
158  
159  
160  
161  
162  
163  
164  
165  
166  
167  
168  
169  
170  
171  
172  
173  
174  
175  
176  
177  
178  
179  
180  
181  
182  
183  
184  
185  
186  
187  
188  
189  
190  
191  
192  
193  
194  
195  
196  
197  
198  
199  
200  
201  
202  
203  
204  
205  
206  
207  
208  
209  
210  
211  
212  
213  
214  
215  
216  
217  
218  
219  
220  
221  
222  
223  
224  
225  
226  
227  
228  
229  
230  
231  
232  
233  
234  
235  
236  
237  
238  
239  
240  
241  
242  
243  
244  
245  
246  
247  
248  
249  
250  
251  
252  
253  
254  
255  
256  
257  
258  
259  
260  
261  
262  
263  
264  
265  
266  
267  
268  
269  
270  
271  
272  
273  
274  
275  
276  
277  
278  
279  
280  
281  
282  
283  
284  
285  
286  
287  
288  
289  
290  
291  
292  
293  
294  
295  
296  
297  
298  
299  
300  
301  
302  
303  
304  
305  
306  
307  
308  
309  
310  
311  
312  
313  
314  
315  
316  
317  
318  
319  
320  
321  
322  
323  
324  
325  
326  
327  
328  
329  
330  
331  
332  
333  
334  
335  
336  
337  
338  
339  
340  
341  
342  
343  
344  
345  
346  
347  
348  
349  
350  
351  
352  
353  
354  
355  
356  
357  
358  
359  
360  
361  
362  
363  
364  
365  
366  
367  
368  
369  
370  
371  
372  
373  
374  
375  
376  
377  
378  
379  
380  
381  
382  
383  
384  
385  
386  
387  
388  
389  
390  
391  
392  
393  
394  
395  
396  
397  
398  
399  
400  
401  
402  
403  
404  
405  
406  
407  
408  
409  
410  
411  
412  
413  
414  
415  
416  
417  
418  
419  
420  
421  
422  
423  
424  
425  
426  
427  
428  
429  
430  
431  
432  
433  
434  
435  
436  
437  
438  
439  
440  
441  
442  
443  
444  
445  
446  
447  
448  
449  
450  
451  
452  
453  
454  
455  
456  
457  
458  
459  
460  
461  
462  
463  
464  
465  
466  
467  
468  
469  
470  
471  
472  
473  
474  
475  
476  
477  
478  
479  
480  
481  
482  
483  
484  
485  
486  
487  
488  
489  
490  
491  
492  
493  
494  
495  
496  
497  
498  
499  
500  
501  
502  
503  
504  
505  
506  
507  
508  
509  
510  
511  
512  
513  
514  
515  
516  
517  
518  
519  
520  
521  
522  
523  
524  
525  
526  
527  
528  
529  
530  
531  
532  
533  
534  
535  
536  
537  
538  
539  
540  
541  
542  
543  
544  
545  
546  
547  
548  
549  
550  
551  
552  
553  
554  
555  
556  
557  
558  
559  
560  
561  
562  
563  
564  
565  
566  
567  
568  
569  
570  
571  
572  
573  
574  
575  
576  
577  
578  
579  
580  
581  
582  
583  
584  
585  
586  
587  
588  
589  
590  
591  
592  
593  
594  
595  
596  
597  
598  
599  
600  
601  
602  
603  
604  
605  
606  
607  
608  
609  
610  
611  
612  
613  
614  
615  
616  
617  
618  
619  
620  
621  
622  
623  
624  
625  
626  
627  
628  
629  
630  
631  
632  
633  
634  
635  
636  
637  
638  
639  
640  
641  
642  
643  
644  
645  
646  
647  
648  
649  
650  
651  
652  
653  
654  
655  
656  
657  
658  
659  
660  
661  
662  
663  
664  
665  
666  
667  
668  
669  
670  
671  
672  
673  
674  
675  
676  
677  
678  
679  
680  
681  
682  
683  
684  
685  
686  
687  
688  
689  
690  
691  
692  
693  
694  
695  
696  
697  
698  
699  
700  
701  
702  
703  
704  
705  
706  
707  
708  
709  
710  
711  
712  
713  
714  
715  
716  
717  
718  
719  
720  
721  
722  
723  
724  
725  
726  
727  
728  
729  
730  
731  
732  
733  
734  
735  
736  
737  
738  
739  
740  
741  
742  
743  
744  
745  
746  
747  
748  
749  
750  
751  
752  
753  
754  
755  
756  
757  
758  
759  
760  
761  
762  
763  
764  
765  
766  
767  
768  
769  
770  
771  
772  
773  
774  
775  
776  
777  
778  
779  
780  
781  
782  
783  
784  
785  
786  
787  
788  
789  
790  
791  
792  
793  
794  
795  
796  
797  
798  
799  
800  
801  
802  
803  
804  
805  
806  
807  
808  
809  
810  
811  
812  
813  
814  
815  
816  
817  
818  
819  
820  
821  
822  
823  
824  
825  
826  
827  
828  
829  
830  
831  
832  
833  
834  
835  
836  
837  
838  
839  
840  
841  
842  
843  
844  
845  
846  
847  
848  
849  
850  
851  
852  
853  
854  
855  
856  
857  
858  
859  
860  
861  
862  
863  
864  
865  
866  
867  
868  
869  
870  
871  
872  
873  
874  
875  
876  
877  
878  
879  
880  
881  
882  
883  
884  
885  
886  
887  
888  
889  
890  
891  
892  
893  
894  
895  
896  
897  
898  
899  
900  
901  
902  
903  
904  
905  
906  
907  
908  
909  
910  
911  
912  
913  
914  
915  
916  
917  
918  
919  
920  
921  
922  
923  
924  
925  
926  
927  
928  
929  
930  
931  
932  
933  
934  
935  
936  
937  
938  
939  
940  
941  
942  
943  
944  
945  
946  
947  
948  
949  
950  
951  
952  
953  
954  
955  
956  
957  
958  
959  
960  
961  
962  
963  
964  
965  
966  
967  
968  
969  
970  
971  
972  
973  
974  
975  
976  
977  
978  
979  
980  
981  
982  
983  
984  
985  
986  
987  
988  
989  
990  
991  
992  
993  
994  
995  
996  
997  
998  
999  
1000  
1001  
1002  
1003  
1004  
1005  
1006  
1007  
1008  
1009  
1010  
1011  
1012  
1013  
1014  
1015  
1016  
1017  
1018  
1019  
1020  
1021  
1022  
1023  
1024  
1025  
1026  
1027  
1028  
1029  
1030  
1031  
1032  
1033  
1034  
1035  
1036  
1037  
1038  
1039  
1040  
1041  
1042  
1043  
1044  
1045  
1046  
1047  
1048  
1049  
1050  
1051  
1052  
1053  
1054  
1055  
1056  
1057  
1058  
1059  
1060  
1061  
1062  
1063  
1064  
1065  
1066  
1067  
1068  
1069  
1070  
1071  
1072  
1073  
1074  
1075  
1076  
1077  
1078  
1079  
1080  
1081  
1082  
1083  
1084  
1085  
1086  
1087  
1088  
1089  
1090  
1091  
1092  
1093  
1094  
1095  
1096  
1097  
1098  
1099  
1100  
1101  
1102  
1103  
1104  
1105  
1106  
1107  
1108  
1109  
1110  
1111  
1112  
1113  
1114  
1115  
1116  
1117  
1118  
1119  
1120  
1121  
1122  
1123  
1124  
1125  
1126  
1127  
1128  
1129  
1130  
1131  
1132  
1133  
1134  
1135  
1136  
1137  
1138  
1139  
1140  
1141  
1142  
1143  
1144  
1145  
1146  
1147  
1148  
1149  
1150  
1151  
1152  
1153  
1154  
1155  
1156  
1157  
1158  
1159  
1160  
1161  
1162  
1163  
1164  
1165  
1166  
1167  
1168  
1169  
1170  
1171  
1172  
1173  
1174  
1175  
1176  
1177  
1178  
1179  
1180  
1181  
1182  
1183  
1184  
1185  
1186  
1187  
1188  
1189  
1190  
1191  
1192  
1193  
1194  
1195  
1196  
1197  
1198  
1199  
1200  
1201  
1202  
1203  
1204  
1205  
1206  
1207  
1208  
1209  
1210  
1211  
1212  
1213  
1214  
1215  
1216  
1217  
1218  
1219  
1220  
1221  
1222  
1223  
1224  
1225  
1226  
1227  
1228  
1229  
1230  
1231  
1232  
1233  
1234  
1235  
1236  
1237  
1238  
1239  
1240  
1241  
1242  
1243  
1244  
1245  
1246  
1247  
1248  
1249  
1250  
1251  
1252  
1253  
1254  
1255  
1256  
1257  
1258  
1259  
1260  
1261  
1262  
1263  
1264  
1265  
1266  
1267  
1268  
1269  
1270  
1271  
1272  
1273  
1274  
1275  
1276  
1277  
1278  
1279  
1280  
1281  
1282  
1283  
1284  
1285  
1286  
1287  
1288  
1289  
1290  
1291  
1292  
1293  
1294  
1295  
1296  
1297  
1298  
1299  
1300  
1301  
1302  
1303  
1304  
1305  
1306  
1307  
1308  
1309  
1310  
1311  
1312  
1313  
1314  
1315  
1316  
1317  
1318  
1319  
1320  
1321  
1322  
1323  
1324  
1325  
1326  
1327  
1328  
1329  
1330  
1331  
1332  
1333  
1334  
1335  
1336  
1337  
1338  
1339  
1340  
1341  
1342  
1343  
1344  
1345  
1346  
1347  
1348  
1349  
1350  
1351  
1352  
1353  
1354  
1355  
1356  
1357  
1358  
1359  
1360  
1361  
1362  
1363  
1364  
1365  
1366  
1367  
1368  
1369  
1370  
1371  
1372  
1373  
1374  
1375  
1376  
1377  
1378  
1379  
1380  
1381  
1382  
1383  
1384  
1385  
1386  
1387  
1388  
1389  
1390  
1391  
1392  
1393  
1394  
1395  
1396  
1397  
1398  
1399  
1400  
1401  
1402  
1403  
1404  
1405  
1406  
1407  
1408  
1409  
1410  
1411  
1412  
1413  
1414  
1415  
1416  
1417  
1418  
1419  
1420  
1421  
1422  
1423  
1424  
1425  
1426  
1427  
1428  
1429  
1430  
1431  
1432  
1433  
1434  
1435  
1436  
1437  
1438  
1439  
1440  
1441  
1442  
1443  
1444  
1445  
1446  
1447  
1448  
1449  
1450  
1451  
1452  
1453  
1454  
1455  
1456  
1457  
1458  
1459  
1460  
1461  
1462  
1463  
1464  
1465  
1466  
1467  
1468  
1469  
1470  
1471  
1472  
1473  
1474  
1475  
1476  
1477  
1478  
1479  
1480  
1481  
1482  
1483  
1484  
1485  
1486  
1487  
1488  
1489  
1490  
1491  
1492  
1493  
1494  
1495  
1496  
1497  
1498  
1499  
1500  
1501  
1502  
1503  
1504  
1505  
1506  
1507  
1508  
1509  
1510  
1511  
1512  
1513  
1514  
1515  
1516  
1517  
1518  
1519  
1520  
1521  
1522  
1523  
1524  
1525  
1526  
1527  
1528  
1529  
1530  
1531  
1532  
1533  
1534  
1535  
1536  
1537  
1538  
1539  
1540  
1541  
1542  
1543  
1544  
1545  
1546  
1547  
1548  
1549  
1550  
1551  
1552  
1553  
1554  
1555  
1556  
1557  
1558  
1559  
1560  
1561  
1562  
1563  
1564  
1565  
1566  
1567  
1568  
1569  
1570  
1571  
1572  
1573  
1574  
1575  
1576  
1577  
1578  
1579  
1580  
1581  
1582  
1583  
1584  
1585  
1586  
1587  
1588  
1589  
1590  
1591  
1592  
1593  
1594  
1595  
1596  
1597  
1598  
1599  
1600  
1601  
1602  
1603  
1604  
1605  
1606  
1607  
1608  
1609  
1610  
1611  
1612  
1613  
1614  
1615  
1616  
1617  
1618  
1619  
1620  
1621  
1622  
1623  
1624  
1625  
1626  
1627  
1628  
1629  
1630  
1631  
1632  
1633  
1634  
1635  
1636  
1637  
1638  
1639  
1640  
1641  
1642  
1643  
1644  
1645  
1646  
1647  
1648  
1649  
1650  
1651  
1652  
1653  
1654  
1655  
1656  
1657  
1658  
1659  
1660  
1661  
1662  
1663  
1664  
1665  
1666  
1667  
1668  
1669  
1670  
1671  
1672  
1673  
1674  
1675  
1676  
1677  
1678  
1679  
1680  
1681  
1682  
1683  
1684  
1685  
1686  
1687  
1688  
1689  
1690  
1691  
1692  
1693  
1694  
1695  
1696  
1697  
1698  
1699  
1700  
1701  
1702  
1703  
1704  
1705  
1706  
1707  
1708  
1709  
1710  
1711  
1712  
1713  
1714  
1715  
1716  
1717  
1718  
1719  
1720  
1721  
1722  
1723  
1724  
1725  
1726  
1727  
1728  
1729  
1730  
1731  
1732  
1733  
1734  
1735  
1736  
1737  
1738  
1739  
1740  
1741  
1742  
1743  
1744  
1745  
1746  
1747  
1748  
1749  
1750  
1751  
1752  
1753  
1754  
1755  
1756  
1757  
1758  
1759  
1760  
1761  
1762  
1763  
1764  
1765  
1766  
1767  
1768  
1769  
1770  
1771  
1772  
1773  
1774  
1775  
1776  
1777  
1778  
1779  
1780  
1781  
1782  
1783  
1784  
1785  
1786  
1787  
1788  
1789  
1790  
1791  
1792  
1793  
1794  
1795  
1796  
1797  
1798  
1799  
1800  
1801  
1802  
1803  
1804  
1805  
1806  
1807  
1808  
1809  
1810  
1811  
1812  
1813  
1814  
1815  
1816  
1817  
1818  
1819  
1820  
1821  
1822  
1823  
1824  
1825  
1826  
1827  
1828  
1829  
1830  
1831  
1832  
1833  
1834  
1835  
1836  
1837  
1838  
1839  
1840  
1841  
1842  
1843  
1844  
1845  
1846  
1847  
1848  
1849  
1850  
1851  
1852  
1853  
1854  
1855  
1856  
1857  
1858  
1859  
1860  
1861  
1862  
1863  
1864  
1865  
1866  
1867  
1868  
1869  
1870  
1871  
1872  
1873  
1874  
1875  
1876  
1877  
1878  
1879  
1880  
1881  
1882  
1883  
1884  
1885  
1886  
1887  
1888  
1889  
1890  
1891  
1892  
1893  
1894  
1895  
1896  
1897  
1898  
1899  
1900  
1901  
1902  
1903  
1904  
1905  
1906  
1907  
1908  
1909  
1910  
1911  
1912  
1913  
1914  
1915  
1916  
1917  
1918  
1919  
1920  
1921  
1922  
1923  
1924  
1925  
1926  
1927  
1928  
1929  
1930  
1931  
1932  
1933  
1934  
1935  
1936  
1937  
1938  
1939  
1940  
1941  
1942  
1943  
1944  
1945  
1946  
1947  
1948  
1949  
1950  
1951  
1952  
1953  
1954  
1955  
1956  
1957  
1958  
1959  
1960  
1961  
1962  
1963  
1964  
1965  
1966  
1967  
1968  
1969  
1970  
1971  
1972  
1973  
1974  
1975  
1976  
1977  
1978  
1979  
1980  
1981  
1982  
1983  
1984  
1985  
1986  
1987  
1988  
1989  
1990  
1991  
1992  
1993  
1994  
1995  
1996  
1997  
1998  
1999  
2000  
2001  
2002  
2003  
2004  
2005  
2006  
2007  
2008  
2009  
2010  
2011  
2012  
2013  
2014  
2015  
2016  
2017  
2018  
2019  
2020  
2021  
2022  
2023  
2024  
2025  
2026  
2027  
2028  
2029  
2030  
2031  
2032  
2033  
2034  
2035  
2036  
2037  
2038  
2039  
2040  
2041  
2042  
2043  
2044  
2045  
2046  
2047  
2048  
2049  
2050  
2051  
2052  
2053  
2054  
2055  
2056  
2057  
2058  
2059  
2060  
2061  
2062  
2063  
2064  
2065  
2066  
2067  
2068  
2069  
2070  
2071  
2072  
2073  
2074  
2075  
2076  
2077  
2078  
2079  
2080  
2081  
2082  
2083  
2084  
2085  
2086  
2087  
2088  
2089  
2090  
2091  
2092  
2093  
2094  
2095  
2096  
2097  
2098  
2099  
2100  
2101  
2102  
2103  
2104  
2105  
2106  
2107  
2108  
2109  
2110  
2111  
2112  
2113  
2114  
2115  
2116  
2117  
2118  
2119  
2120  
2121  
2122  
2123  
2124  
2125  
2126  
2127  
2128  
2129  
2130  
2131  
2132  
2133  
2134  
2135  
2136  
2137  
2138  
2139  
2140  
2141  
2142  
2143  
2144  
2145  
2146  
2147  
2148  
2149  
2150  
2151  
2152  
2153  
2154  
2155  
2156  
2157  
2158  
2159  
2160  
2161  
2162  
2163  
2164  
2165  
2166  
2167  
2168  
2169  
2170  
2171  
2172  
2173  
2174  
2175  
2176  
2177  
2178  
2179  
2180  
2181  
2182  
2183  
2184  
2185  
2186  
2187  
2188  
2189  
2190  
2191  
2192  
2193  
2194  
2195  
2196  
2197

Table 6. Fiber composition and protein recoverability upon accessing grass and potato tops on a semitechnical scale using a Sands Disk Refiner.

Raw material	composition raw material			disk		through-put (kg d.m./hour)	composition fiber		protein recoverability
	d.m.	ash	N	Plate resistance	disk distance		ash	N	
	(g/kg fresh)	(g/kg d.m.)	(g/kg d.m.)		mm	(kg d.m./hour)	(g/kg d.m.)	(g/kg dm)	(%)
cultivat. grass	154	91	19.3	high	0.4	-	13	5	91
cultivat. grass	142	183	36.1	high	0.10	39	31	14	87
"	"	"	"	high	0.50	55	27	15	86
"	"	"	"	high	1.00	104	38	15	86
"	"	"	"	low	0.05	157	49	14	87
"	"	"	"	low	0.10	135	41	14	87
"	"	"	"	low	0.50	139	54	15	86
"	"	"	"	low	1.00	211	82	20	82
natural grass	215	138	12.1	low	0.10	-	41	6	84
potato tops	104	342	23.5	high	0.20	-	473	15.2	-
"	119	344	27.0	low	0.20	-	374	19.0	-

## Process diagrams for refining grass

### Pretreatment

5

The appended process diagrams (see Figures 8 to 10) start from the supply of chopped grass as is also conventional in the processing of grass and lucerne in herbage dryers. Normally, the chopping length is in the order of magnitude of a few centimeters, but it can also be longer or shorter. For the  
10 refiner test, fresh grass was pre-comminuted in a Pierret guillotine cutter to 6 mm particle length, in other words, very short. Presumably, such a short length is not requisite; refining or fiberizing squeezed grass (of a particle length of presumably a few centimeters) did not present any problems.

15 Washing

A washing step will probably be necessary in practice to remove sand and thereby reduce equipment wear and enable a cleaner product yield. This washing step, however, may be skipped if sand and other contaminants are  
20 not present.

### Sulfite addition

Addition of sulfite may be necessary, but need not be so, to prevent  
25 undesirable complexing between proteins and polyphenols. On the basis of past experiences regarding the processing of grass juice, it is known that such complex formation reduces the nutritive values of grass proteins. The circumstances during refining, however, may be different. A rapid temperature rise during refining may instantly stop enzymatic activity  
30 (blanching effect) and inhibit formation of polyphenols.

### Refining: basic diagram (Fig. 8)

Refining grass is in principle possible with and without liquid addition during refining. In a first test, with fresh grass (15% dry matter), the process did not proceed readily without generous admixture of water to a dry matter percentage of about 2%. The necessity of liquid addition is probably partly dependent on the type of refiner and the nature of the grass (fibrousness). Pressed grass (26% dry matter) could be refined without water addition. If, and if so, how much water is admixed, has consequences for the temperature rise during refining, and therefore for the extent of protein denaturation and hence for the subsequent steps in the process.

The basic diagram includes, after refining, the process steps: screening out the fiber, heat coagulating the refiner liquid followed by separation of the protein cake by means of a decanter and evaporation of the deproteinized liquid. Two extreme variants of this basic diagram are conceivable: one with a minimal addition of liquid during refining and one with ample addition of liquid. The basic diagram is then changed to variant A (Figure 9) and variant B (Figure 10), respectively.

### Refining: variant A (Figure 9)

Upon minimal addition of return liquid, possibly a substantial temperature rise will occur during refining: in the test with pressed grass to above 70°C. Protein coagulation and pasteurization will then occur already during refining and possibly a separate coagulation step may then be skipped. In that case, the process diagram is simplified to refining - screening - decanting - evaporation: see variant A on basic diagram.

Refining: variant B (Figure 10)

Variant B: In case of ample addition of return liquid, the temperature rise during refining can remain limited: in the test with fresh grass to about 35° C. As a result, presumably, a part of the protein can remain in solution. In that case, after refining, two alternative routes are conceivable. The simplest one is, after screening out the fiber, to heat-coagulate the liquid and decant. In that case, one protein cake is formed and a deproteinized liquid that can be evaporated (see the basic diagram). A more complex route (variant B) comprises, after screening out the fiber, initial decanting whereby a crude protein cake is obtained (crude, i.e. with admixture of finely divided parenchymal cell walls that pass the screen), followed by heat-coagulation and decanting again. In this second decanting step, a purer protein cake is obtained.

#### Screening out the fibers

For the purpose of screening out the fibers, centriscreens can be employed, as known to those skilled in the art for separating potato fiber. In the test, an inclined screen was used, having stretched onto it a wire gauze with openings of 140 \* 140 microns. On a lab scale, a screen with a hole diameter of 850 and 250 microns was used. Experiences with it are that most fibers can be separated on a relatively coarse screen. The finer fiber fraction can be added to the total fiber fraction or, via enzymatic deliquescence, to the molasses, concentrate or juice stream.

#### Washing and drying of fiber

The fiber that is separated by screening may be contaminated with dissolved and suspended substance. Accordingly, washing with



deproteinized return liquid is then necessary, followed by moisture removal through pressing/centrifugation and drying.

#### Drying protein cake

5

The protein-rich cake which is separated through decanting can be dried in the same manner as is known to those skilled in the art for, for instance, potato protein. In case of the presence of a relatively high lipid fraction, addition of an antioxidant product has an improving effect.

10

#### Evaporation of deproteinized liquid

The deproteinized liquid can be evaporated to form a sugar-rich syrup.

#### 15 Extended procedure

The basic diagram can be expanded to include processes for the purpose of further refining the crude protein cake. One possible addition is enzymatic deliquescence of the parenchymal cell walls in the crude protein cake. The  
20 sugars which this yields can, for instance, be added to the molasses, concentrate or juice stream.

CLAIMS

1. A method for separating components from vegetable material which comprises at least leaf and/or stem parts, characterized in that the material is at the least partly fiberized, and subsequently is separated into a fiber fraction and a juice stream, such that the fiber fraction principally comprises relatively firm tissues such as epidermis, sclerenchyma and vascular bundles, and the juice stream principally comprises soft tissues such as parenchyma, and cytosol.
2. A method according to claim 1, wherein the juice stream comprises chloroplasts.
3. A method according to claim 1 or 2, wherein the material is mechanically fiberized.
4. A method according to claim 3, wherein the material is fiberized by means of a refiner.
5. A method according to any one of claims 1-4, wherein the fiber fraction is separated from the juice stream through screening.
6. A method according to any one of claims 1-5, wherein the vegetable material originates from a cultivated crop.
7. A method according to claim 6, wherein the cultivated crop belongs to the family of grasses.
8. A fiber fraction obtained by a method according to any one of claims 1-7.
9. Use of a fiber fraction according to claim 8.
10. Use of a fiber fraction according to claim 9 for the production of energy or for the production of cardboard and/or paper.
11. A juice stream obtained by a method according to any one of claims 1-7.

12. A juice stream according to claim 11, which contains more than 55%, preferably more than 75%, preferably more than 90% of the crude protein of the vegetable material.
13. Use of a juice stream according to claim 11 or 12.
14. Use of a juice stream according to claim 11 or 12 for the production of food.
15. Use of a juice stream according to claim 11 or 12 for recovering or purifying at least a content substance.
16. An apparatus for carrying out a method according to any one of claims 1-7.
17. An apparatus according to claim 16 which comprises at least a refiner.
18. An apparatus in which a method according to any one of claims 1-7 is carried out.

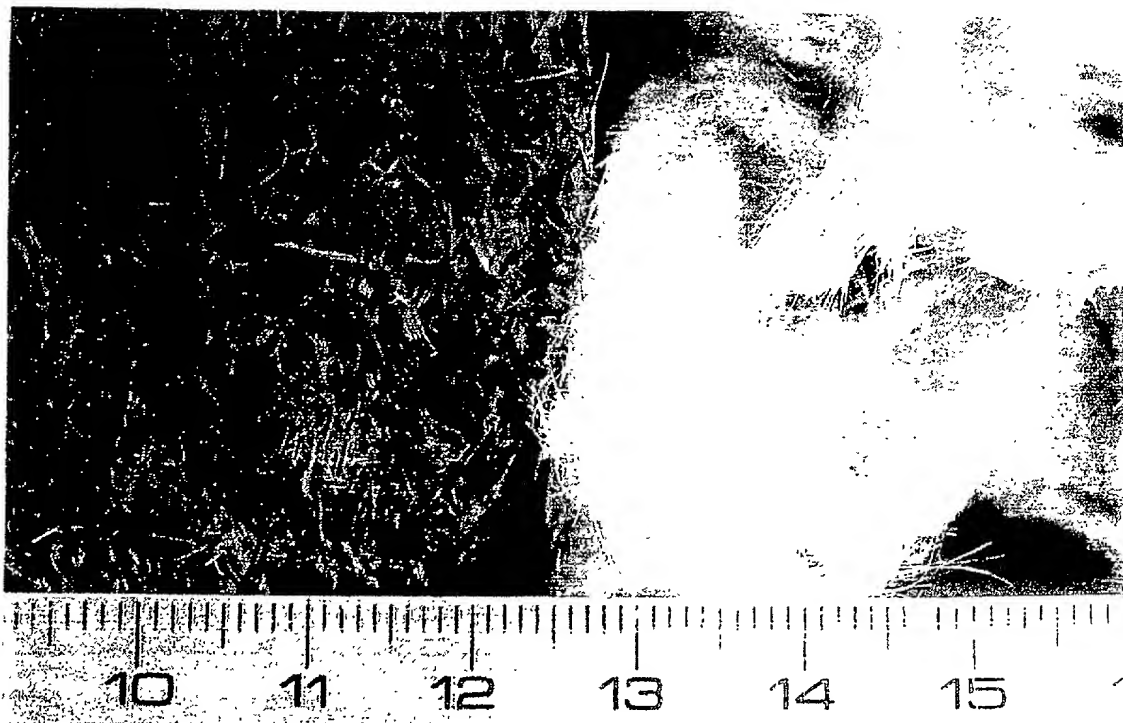


Fig. 1

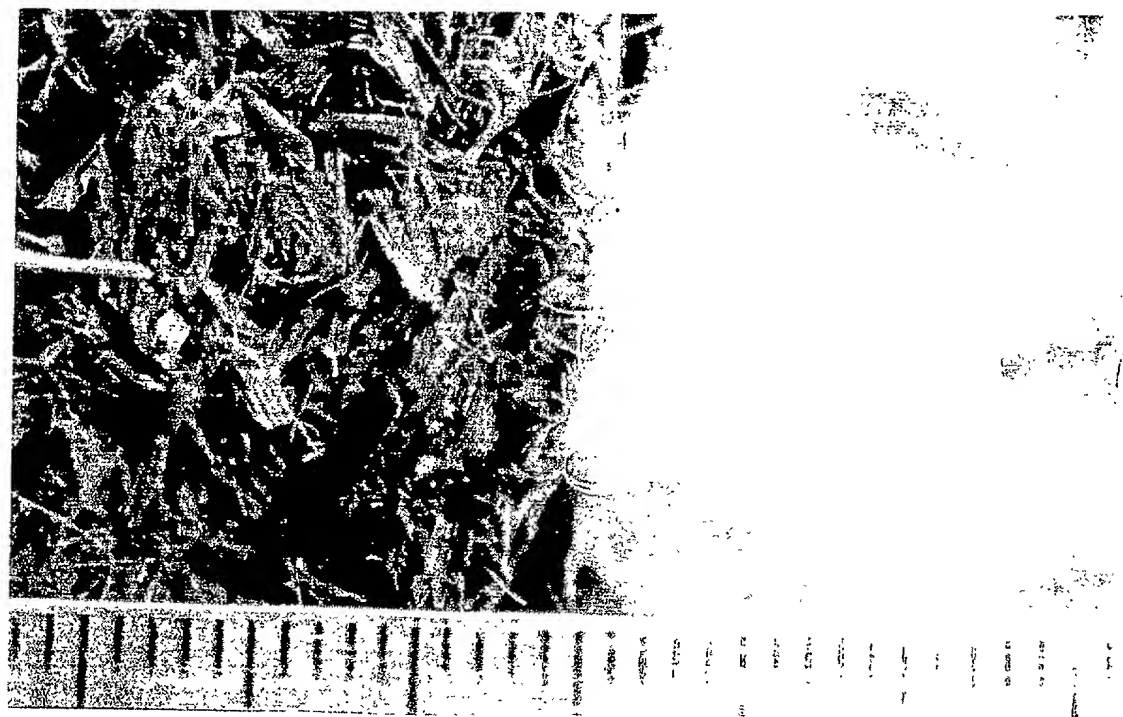


Fig. 2

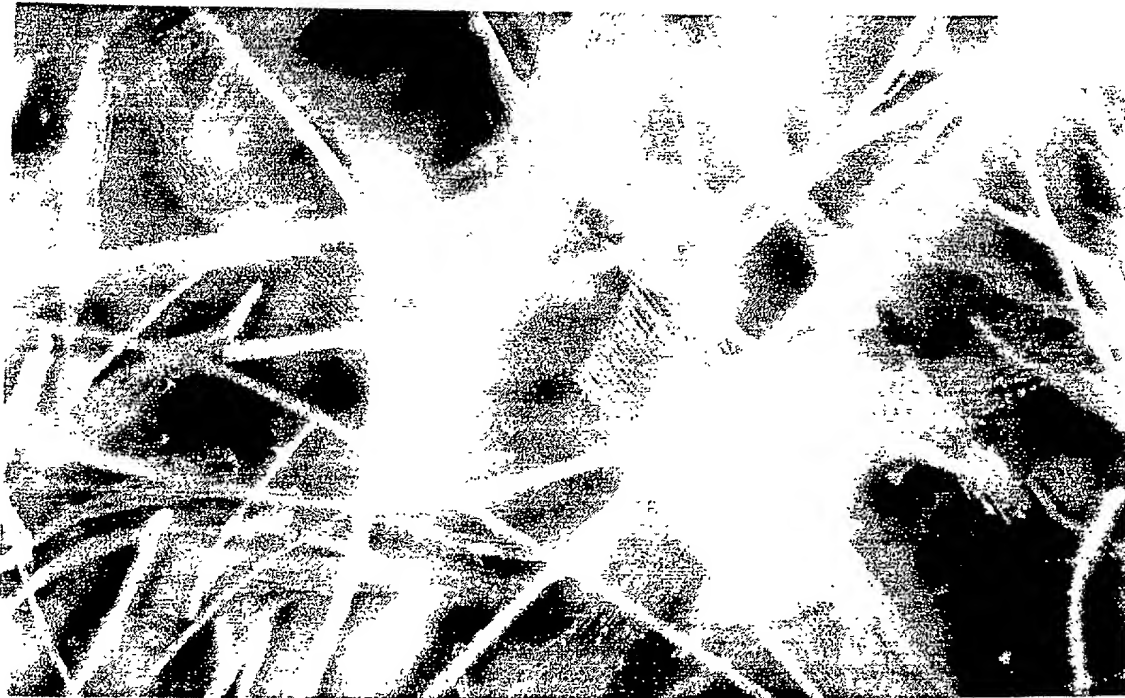


Fig. 3

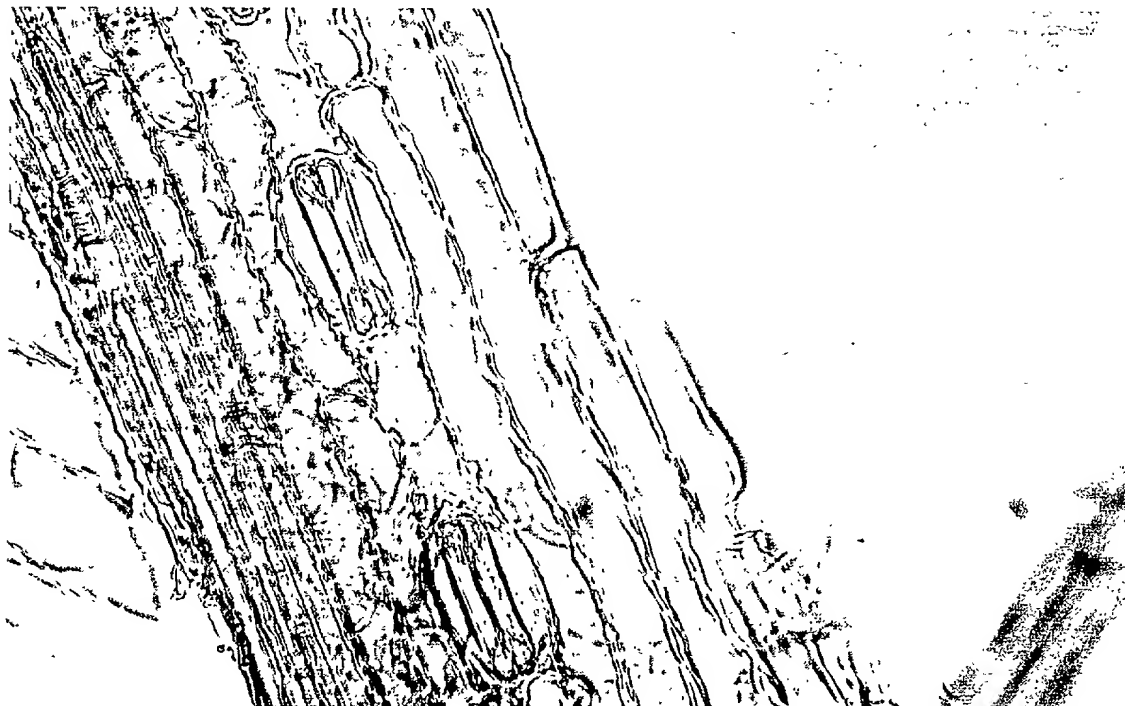


Fig. 4

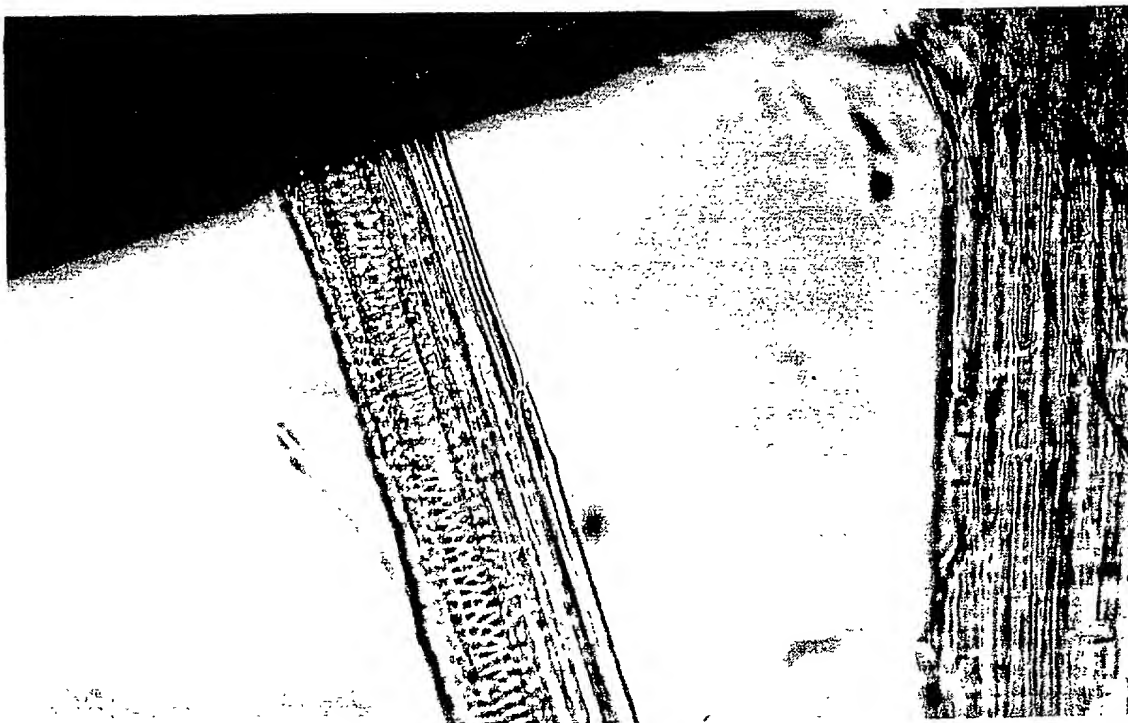


Fig. 5

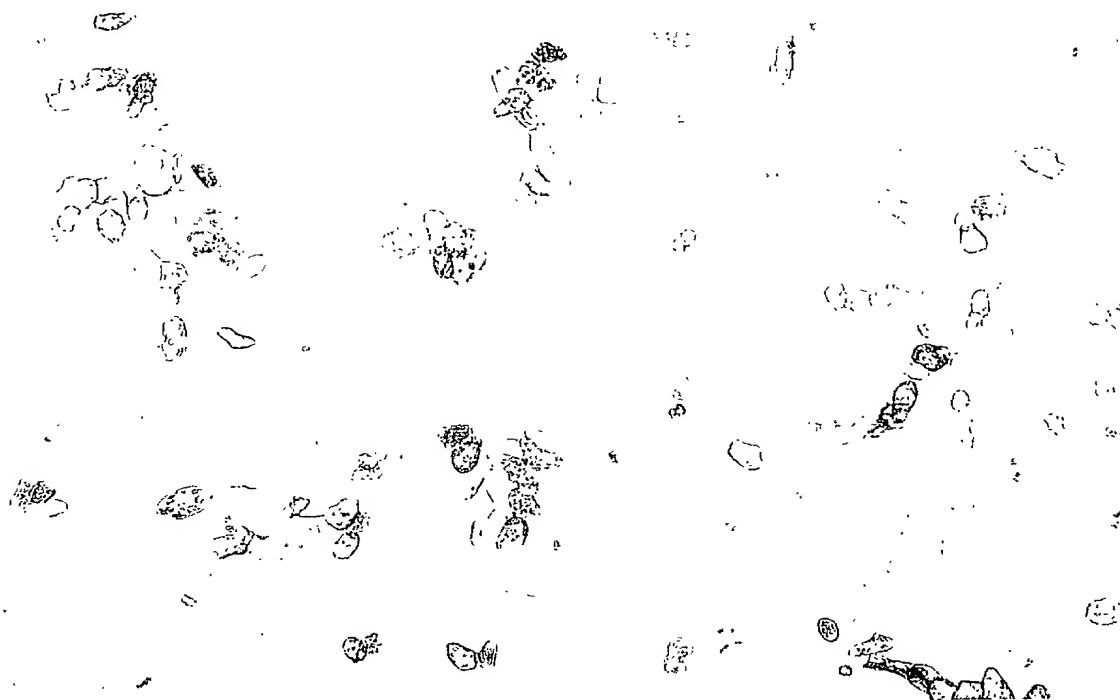
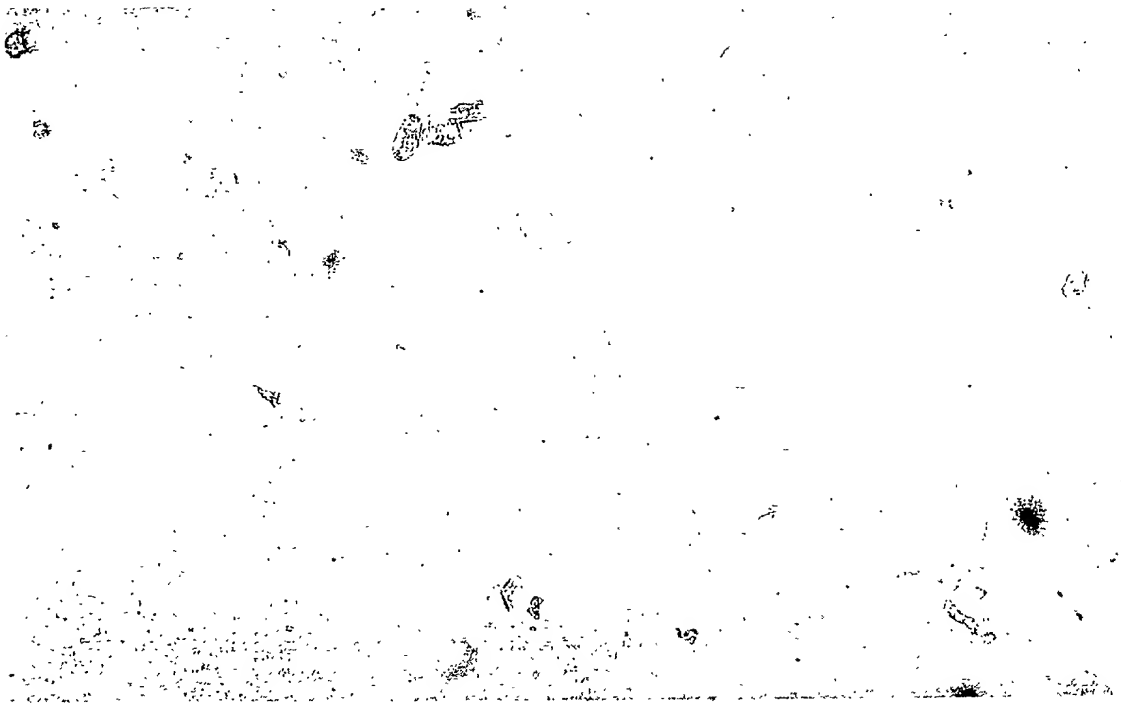


Fig. 6



**Fig. 7**

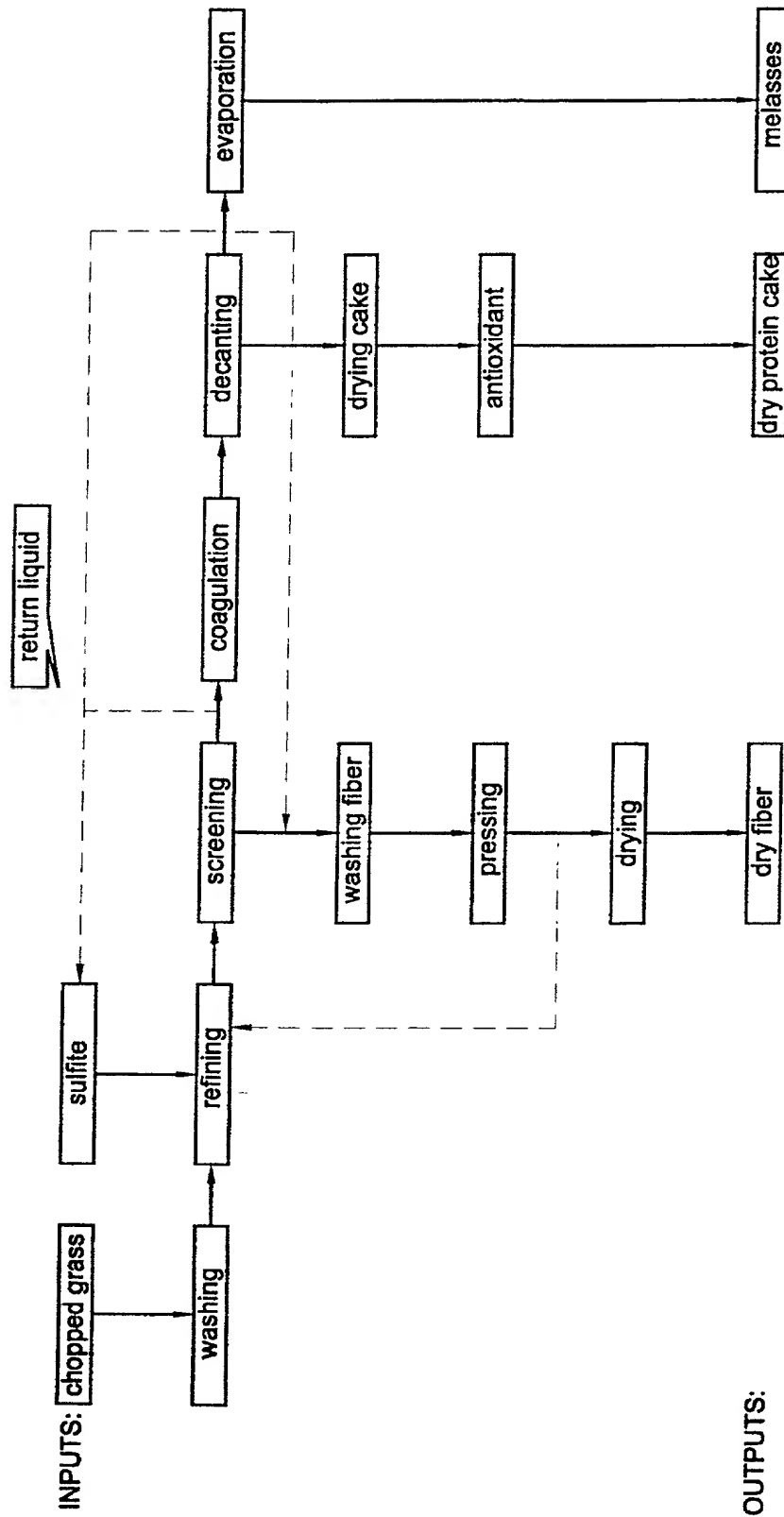


Fig. 8



6/7

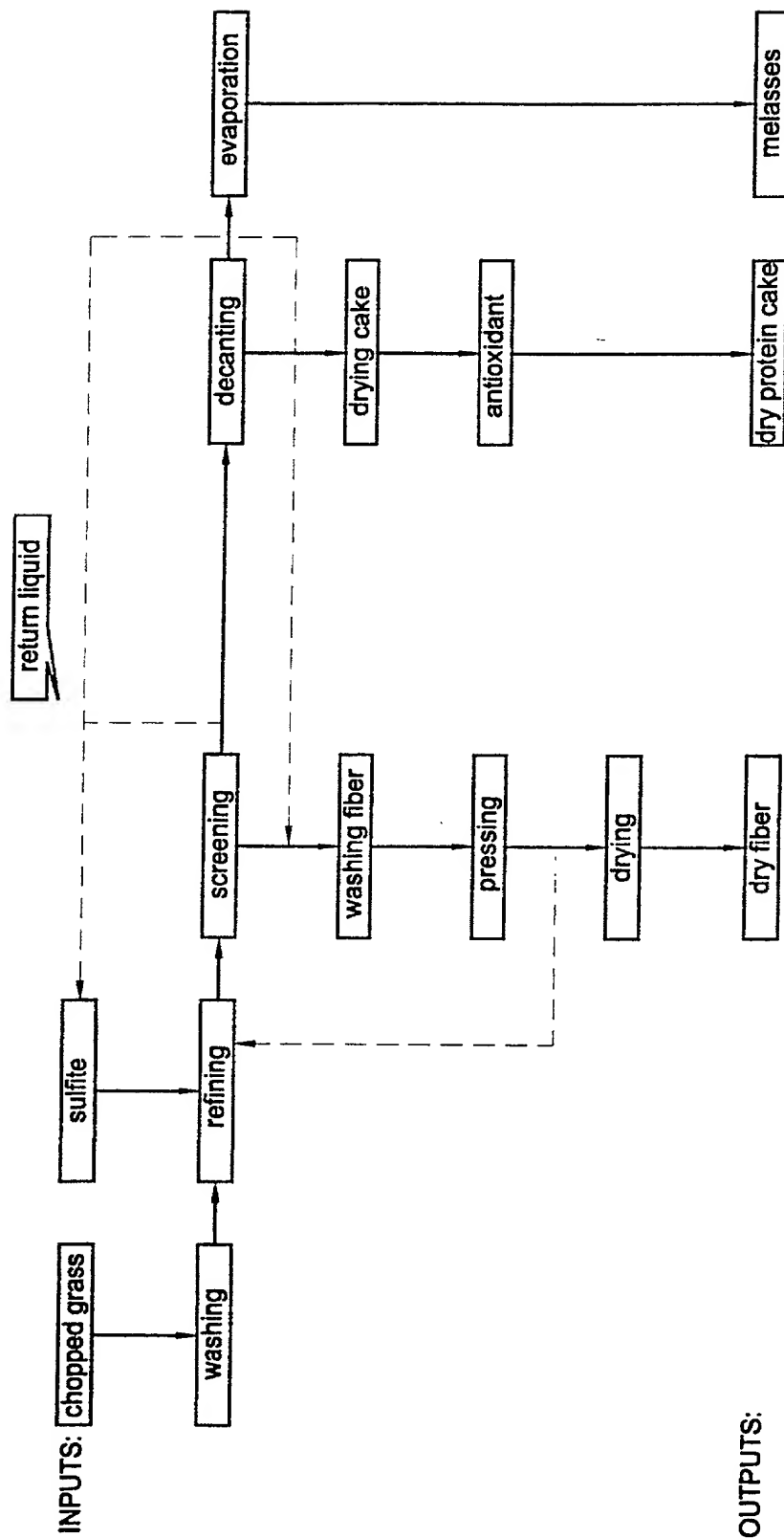


Fig. 9

7/7

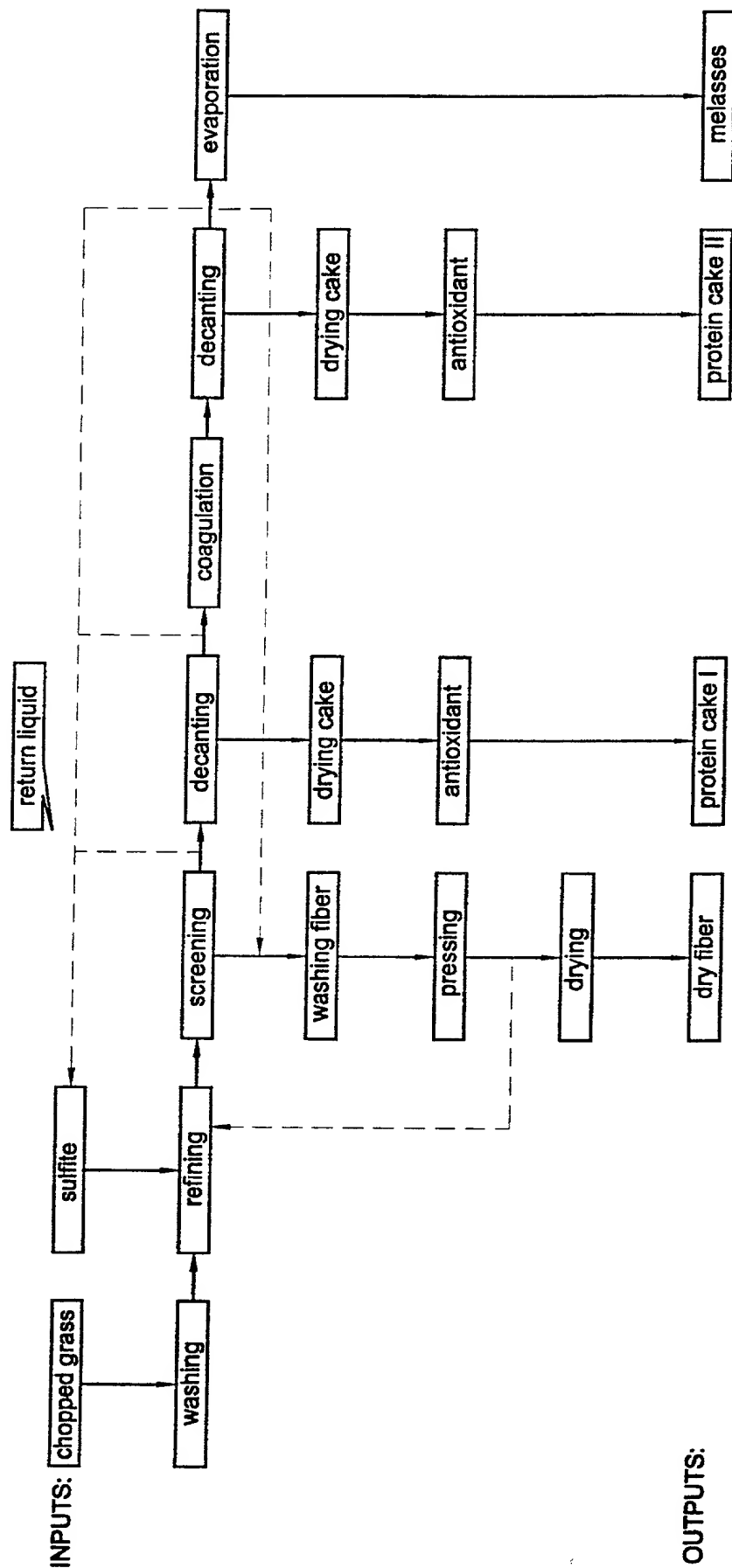


Fig. 10

**Declaration and Power of Attorney Patent Application  
(Design or Utility)**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: 'Accessing leaf and/or stem parts of plants'

the specification of which

- ☐ is attached hereto  
x was filed on June 25, 2001 ✓ as application serial no. 09/869,409 ✓  
and or PCT International Application number PCT/NL99/00804 and was amended  
on (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information know to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or 35 U.S.C. §365(b) of any foreign application(s) for patent or inventor's certificate, or 35 U.S.C. §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate of PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)		
Number 1010975 ✓	Country NL ✓	Day/Month/Year Filed 06-01-1999 ✓
Number	Country	Day/Month/Year Filed
Number	Country	Day/Month/Year Filed

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

Prior Provisional Application(s)	
Serial Number	Day/Month/Year Filing Date
Serial Number	Day/Month/Year Filing Date
Serial Number	Day/Month/Year Filing Date

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s), or under 35 U.S.C. §365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

Prior U.S. or International Application(s)		
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

## Power of Attorney

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

### Attorney


### Registration Number

Charles R. Hoffmann	<u>24,102</u>
Ronald J. Baron	<u>29,281</u>
Gerald T. Bodner	<u>30,449</u>
A. Thomas Kammer	<u>28,226</u>
Irving N. Feit	<u>28,601</u>
Alan M. Sack	<u>31,874</u>
Algis Anilionis	<u>36,995</u>
Gregory W. Bachmann	<u>41,593</u>
Anthony E. Bennett	<u>40,910</u>
James F. Harrington	<u>P-44,741</u>
Glenn T. Henneberger	<u>36,074</u>
Richard LaCava	<u>41,135</u>
Kevin E. McDermott	<u>35,946</u>
Robert C. Morriss	<u>42,910</u>
Samir R. Patel	<u>P-44,998</u>
R. Glenn Schroeder	<u>34,720</u>
Susan A. Sipos	<u>43,128</u>
Roderick S.W. Turner	<u>38,639</u>
Steven T. Zuschlag	<u>43,309</u>

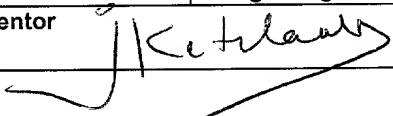
I hereby authorize them or others whom they may appoint to act and rely on instructions from and communicate directly with the person/organization who/which first sends this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instructed otherwise.

Please direct all correspondence in this case to at the address indicated below:


Ronald J. Baron  
Hoffman & Baron, L.L.P.  
6900 Jericho Turnpike  
Syosset, New York 11791

Full Name of Sole or First Inventor		
Family Name <u>Hulst</u>	First Given Name <u>Anne</u>	Second Given Name <u>Coenraad</u>
Residence and Citizenship		
City of Residence <u>Gieten</u> <i>NLX</i>	State or Country of Residence <u>The Netherlands</u>	Country of Citizenship <u>The Netherlands</u> ✓
Post Office Address		
Street Address <u>Julianalaan 57</u>	City <u>Gieten</u>	State & Zip Code or Country <u>9461 BS</u>
Signature of Inventor 		Date <u>31-7-2001</u>

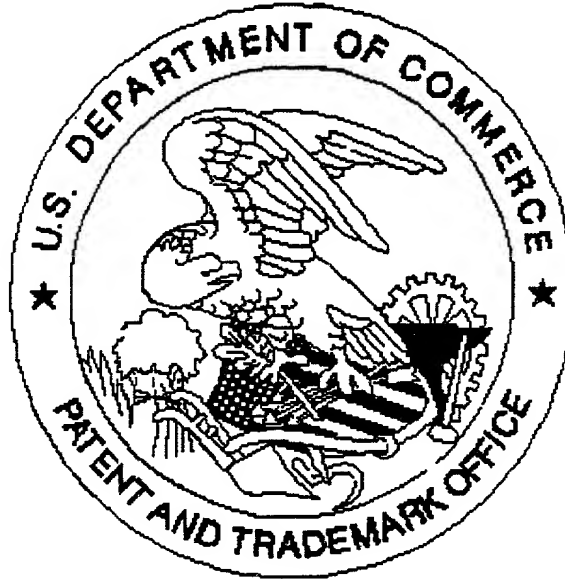
2-00

Full Name of Second Inventor, if any		
Family Name <u>Ketelaars</u>	First Given Name <u>Jan</u>	Second Given Name <u>Josef, M, H,</u>
Residence and Citizenship		
City of Residence <u>Wageningen</u> NLX	State or Country of Residence <u>The Netherlands</u>	Country of Citizenship <u>The Netherlands</u> ✓
Post Office Address		
Street Address <u>Torckpark 40</u>	City <u>Wageningen</u>	State & Zip Code or Country <u>6701 ED</u>
Signature of Inventor 		Date <u>31-7-2001</u>

3-00

Full Name of Third Inventor, if any		
Family Name <u>Sanders</u>	First Given Name <u>Johan</u>	Second Given Name <u>Pieter, M.</u>
Residence and Citizenship		
City of Residence <u>Groningen</u> NLX	State or Country of Residence <u>The Netherlands</u>	Country of Citizenship <u>The Netherlands</u> ✓
Post Office Address		
Street Address <u>Saaksumberg 1</u>	City <u>Groningen</u>	State & Zip Code or Country <u>9722 WL</u>
Signature of Inventor 		Date <u>31-7-2001</u>

United States Patent & Trademark Office  
Office of Initial Patent Examination -- Scanning Division



Application deficiencies found during scanning:

☐ Page(s) \_\_\_\_\_ of \_\_\_\_\_ were not present  
for scanning. (Document title)

☐ Page(s) \_\_\_\_\_ of \_\_\_\_\_ were not present  
for scanning. (Document title)

Scanned copy is best available. Drawing Fig 1, 3, 5, 7, are dark